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ACCEPTABILITY AND ASSESSMENT OF PHYSICOCHEMICAL COMPOSITION AND ANTIOXIDANT POTENTIAL OF MAIZE-WATERMELON SEED FLOUR BLENDS AND MASA

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Abstract
Masa is a fermented bread-like product made from millet, maize or rice flour in Nigeria. It is round in shape with brown smooth body and crippling edges. .Cereals are deficient in the essential amino acid lysine and complementation with plant proteins has been reported to improve their protein quality. Thus, the present study was aimed at enriching maize-based masa with a lesser-known protein source, watermelon seed flour. Maize-watermelon seed flour blends (100:0, 95:5, 90:10, 85:15) were subjected to physicochemical (pH, total titratable acidity, proximate), mineral, phytochemical and antioxidant analyses while sensory qualities of masa produced from the flour blends were determined. The pH decreased from 6.4 to 5.4, while total titrayable acidity TTA (2.3% – 3.7% lactic acid), protein (1.68% to 4.43%), fat (11.60% to 18.01%) and ash contents (0.96% to 2.98%) of maize–watermelon seed flour increased significantly ($p<0.05$) with addition of watermelon seed flour. Similarly, potassium, magnesium, copper and manganese increased significantly ($p<0.05$). The free radical scavenging (DPPH) (39.02%-60.74%), flavonoid (621.02 mg/g-1526 mg/g), phenol (604.76 mg/g-670.46 mg/g) and tannin (130.97 mg/g-314.30 mg/g) were also found to increase with increase in watermelon seed flour. Results of consumer acceptability test revealed that masa made from 95:5 and 90:10 maize-watermelon seed blends did not differ significantly ($p>0.05$) from the control (100% maize). Hence, the 90:10 maize-watermelon seed flour blend may be best recommended based on its comparable sensory attributes and higher nutritional composition, phenolic content and free radical scavenging activity.

Introduction

The high level of starch and good digestibility makes maize one of the staple food for infants, lactating mothers, the elderly and as well for animal feed (Darrah, 2003). Maize is employed in the production of flour, tortillas, snacks, masa, steamed products, and breakfast cereals (Rooney and Serna-Saldivar, 1987). Masa is a fermented bread-like product made from maize, millet or rice flour and commonly consumed in Northern Nigeria. It has brown smooth body with crippling edges. (Ayo *et al*., 2008). Masa is prepared as a variety in cereal food products, served either as breakfast, snack and sometimes eaten with local soup. The quality of maize proteins is poor because they are deficient in the essential amino acids; lysine and tryptophan and this determines the nutrient content of masa produced from it. Protein content of 4.76%-4.9% and fat (0.74%-5.76%) was reported for masa made from maize (Rosentrater et al., 1999).

Watermelon fruit contains many smooth compressed seeds and have been found to be rich in protein and oil (Oyeleke *et al*., 2012; Ifesan and Ebosele, 2017) antioxidants, minerals and phytochemicals (El-Adawy and Taha, 2001; Hayashi *et al*., 2005). Watermelon seed contain 1.01 g/100g (Egbuonu et al., 2015) and 3.15 g/100g (Ifesan and Ebosele, 2017) lysine which is the limiting factor in maize. Addition of cowpea, groundnut and soybean flour into masa to improve the nutritional qualities has been reported (Nkama and Malleshi, 1998; Ayo *et al*., 2008). Watermelon seed is discarded after eating the rind and it may constitute nuisance to the environment if not properly disposed. Since the limiting amino acid in maize is found in watermelon seed, this work was carried out to investigate the chemical composition and consumer acceptability of masa enriched with water melon seed flour.

Materials and Methods

Sample Collection

Maize grain (*Zea mays*) and watermelon seed (*Citrullus lanatus*) were purchased from Sasha market in Akure, Ondo State. All chemical reagent used were of analytical grade.

Production of Watermelon Seed Flour

The watermelon seed was processed using the method of Ifesan and Ebosele (2017). Watermelon seed was removed manually from the watermelon pod, washed, dried in the hot air oven at 50°C and milled into flour. Watermelon and melon seed that people use as thickner in soup are in the same family Cucurbitaceae so there is not likely to be any anti-nutrient that can pose health risk to the consumer. And besides the watermelon seed together with the maize were subjected to heat treatment during masa production. Maize-watermelon seed flour blends were formulated using different graded levels of maize and watermelon seed flours as shown in Table 1.

Preparation and Frying of Masa

Masa was prepared from the control (100% maize flour) and the flour blends n Table 1 using the method of Ayo *et al.* (2008). The maize grain was cleaned, washed, dried in hot, milled, and sieved into grits and flour. The maize grits was cooked, cooled down (32°C), mixed with the appropriate gram of watermelon seed flour (Table 1) and little quantity of water was added to form a batter. The resulting batter was inoculated with 1.0% yeast and allowed to ferment overnight for 12 h at room temperature of about (32°C). The batter was then diluted with 20% *trona solution*. Pinch of salt and 6% sugar was added to the batter, stirred vigorously using a mortar and pestle to incorporate air and fried in a local clay pot with individual cuplike hollow in which oil has been added. The batter was placed inside the oil and allowed to fry for some minutes to produce masa.

Table 1: Formulation of Maize-watermelon Seed Flour For Masa Production

Flour blends	% Maize flour/grits	% Watermelon seed flour
MW1	100	0
MW2	95	5
MW3	90	10
MW4	85	15

Determination of Physicochemical Properties of Maize-watermelon Seed Masa Dough

The pH values of the fermented samples were determined using a pH meter (Crison Basic, model 20) according to approved method 44-19 (AACC, 2000). Ten grams of sample were placed in a beaker containing 100 ml of distilled water (10% w/v slurry) and stirred for 15 min to homogenize the sample. The resulting suspension was left to stand for 15 min, and the pH level was read in the supernatant liquid. Ten-milliliter aliquots of filtrates of the slurry were titrated against 0.1 N NaOH standard solution to determine the total titratable acidity. Crude protein, lipid, crude fibre, moisture and ash content were determined in accordance with the standard methods of AOAC (2005). Carbohydrate was determined by difference.

Determination of Mineral Element Composition of Maize-watermelon Seed Flour

Two grams of sample was placed in a crucible, ashed in a muffle furnace at 550°C for 5 h and transferred into the desiccator to cool. The ashed sample was dissolved with 1ml nitric acid and 1 ml HCl and made up to 100 ml. This was used to analyze for Magnesium, sodium, and calcium, The atomic absorption spectrophotometer was used to determine these elements (AOAC, 2005).

Determination of Phytochemical, Antinutrient and Antioxidant Properties of Flour Blends Used for Masa Production

Phytochemical (phytate and tannin) and antinutrient (oxalate) content of the flour blends used in masa production were determined following standard methods. (Chitravadivu *et al*., 2009). About 0.2 g of masa sample was weighed into 10 ml of 70% aqueous acetone and was properly covered. The bottles were put in an ice bath shaker for 2 h at 30°C. Each solution was then centrifuged and the supernatant stored in ice. About 0.2 ml of each solution was pipetted into the test tube and 0.8 ml of distilled water was added. Standard tannic acid solutions were prepared and 0.5 ml of Folin Ciocateau reagent was added to both samples and standard followed by 2.5 ml of 20% Na_2CO_3. The solutions were vortex and allowed to incubate for 40 min at room temperature, its absorbance was read at 725 nm against a reagent blank.

Oxalate was determined by soaking 1 g of sample in 75 ml of 1.5M H_2SO_4 for 1 h and then filtered through a No 1 Whatman filter paper. About 25 ml was taken out of the filtrate, poured inside a conical flask and titrated hot (80-90°C) against 0.1M $KMnO_4$ until a pink colour that persist for 15 sec was obtained. For phytate determination, about 4 g of sample was soaked with 100 ml of 2% HCl for 3 h and then filtered through a No 1 Whatman filter paper. Twenty five milliliter of the filtrate was taken and 5 ml of 0.3% of ammonium thiocyanate solution was added as indicator, after which 53.5 ml of distilled water was added to give it the proper acidity. This was titrated against 0.00566 g per milliliter of standard iron (lll) chloride solution that contained about 0.00195 g of iron per milliter until a brownish yellow colouration persists for five minutes.

Total phenol content was determined according to the method of Singleton *et al.* (1999). Free radical scavenging activity of samples against 1,1-diphenyl-2 picrylhydrazyl (DPPH) was evaluated as described by Hutadilok-Towatana *et al*. (2006). Total flavonoid content of flour blends was determined using a slightly modified method reported by Varghese *et al*. (1995). About 0.2 ml of the extract from masa dough was added to 0.3 ml of 5% $NaNO_3$. After 5 min, 0.6 ml of 10% $AlCl_3$ was added and later, 2 ml of 1M NaOH was added to the mixture followed by the addition of 2.1 ml of distilled water. Absorbance was read at 510 nm against the reagent blank and flavonoid content was expressed as mg rutin equivalent.

Sensory Evaluation of Masa Produced from Maize-watermelon Seed Flour Blends

A total of 21 assessors evaluated the masa samples for attributes of appearance, color, texture, taste, and overall acceptability using a 9-point hedonic scale (Solomakos *et al*. 2008).

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using the Statistical Package for Social Sciences (windows????). Comparison of means was carried out by Duncan's multiple range test (Steel and Torrie, 1980). . All analyses except otherwise stated, were carried out in triplicate. Means were separated using one-way analysis of variance and significant differences were determined using Duncan's new multiple range test at $p < 0.05$.

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Results and Discussion

Physicochemical properties of Maize-watermelon Seed Flour Blends

The flour samples had a pH range between 6.3 – 6.5 at the initial stage and reduced to 5.4 – 5.5 after 12 h (Figure 1). It was observed that 100% maize (MW1) had the lowest total titratable acidity TTA value (1.90 g/l - 2.30 g/l) and it increased with addition of watermelon seed resulting in 3.10 g/l -3.70 g/l in MW4 (85:15) flour blend (Figure 2). It may be explained that production of organic acid during fermentation resulted in reduction of pH and increased TTA. Total titratable acidity is the measure of the lactic acid produced by the lactic acid bacteria in the medium and signifies the reduction in pH or acidity of the fermenting medium (Doyle *et al.,* 2001). This result is similar to previous reports where rice was supplemented with soybean for masa production (Ezeama and Ihezie, 2006).

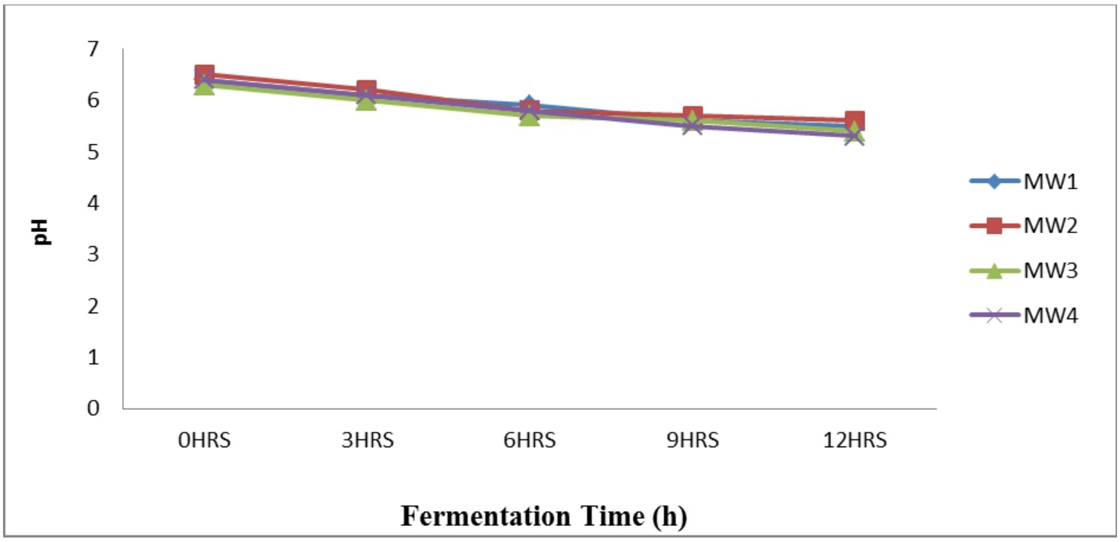

Figure 1: pH of the maize-water melon seed fermented batter.

MW1 = 100% maize; MW2 = 95% maize + 5% watermelon seed; MW3 = 90% maize + 10% watermelon seed; MW4 = 85% + 15% watermelon seed.

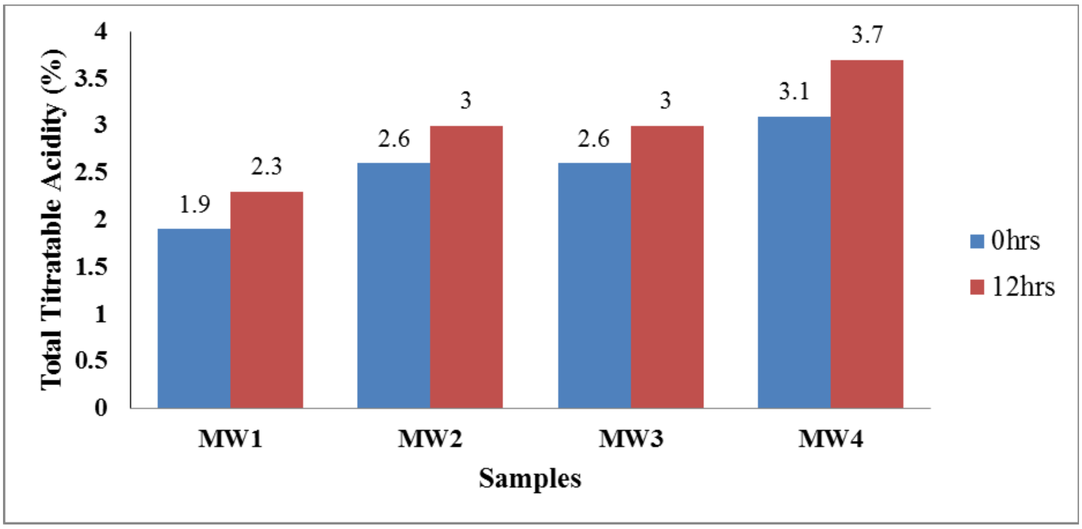

Figure 2: Total titratable acidity (% lactic acid) of the fermented batters from 0 h and 12 hours
Mw1 = 100% maize; MW2 = 95% maize + 5% watermelon seed; MW3 = 90% maize + 10% watermelon seed; MW4 = 85% + 15% watermelon seed.

Addition of watermelon seed flour has significant ($p<0.05$) effect on the proximate composition of the flour blends (Table 2). The moisture content of the flour decreased significantly ($p<0.05$) with increase in watermelon seed from 11.37% in 100% maize (MW1) to 8.07% in 85% maize:15% watermelon seed flour (MW4). The protein content (1.89%-4.82%), fat (13.09%-13.09%) and ash (1.08%-3.24%) of the flour samples were found to increase significantly ($p<0.05$) with watermelon seed substitution. This may be due to the high protein, fat and mineral content of watermelon seed (Mabaleha *et al.,* 2007; Varghese *et al*., 2013). However, crude fibre of the blends decreased with increase in watermelon seed flour from 3.64% in 100% maize to 1.62% in 85%:15% flour (MW4) which may be as a result of the removal of the hull after milling.

Table 2: Proximate Composition (%) Of Maize–watermelon Seed Flour Blends

Table 2: Proximate composition (%) of maize–watermelon seed flour blends

Samples	Moisture	Ash	Crude fibre	Protein	Fat	Carbohydrate
MW1	11.37 ± 0.00^{a}	0.96 ± 0.01^{d}	3.23 ± 0.35^{a}	1.68 ± 0.14^{d}	11.60 ± 0.14^{d}	71.17 ± 0.62^{a}
MW2	11.06 ± 0.14^{a}	1.95 ± 0.21^{c}	2.78 ± 0.14^{a}	1.98 ± 0.07^{c}	15.03 ± 0.35^{c}	67.21 ± 0.09^{b}
MW3	8.28 ± 0.15^{b}	2.93 ± 0.02^{b}	2.69 ± 0.15^{a}	3.53 ± 0.04^{b}	17.02 ± 0.28^{b}	65.57 ± 0.01^{c}
MW4	8.07 ± 1.64^{b}	2.98 ± 0.14^{a}	1.49 ± 0.00^{b}	4.43 ± 0.01^{a}	18.01 ± 0.01^{a}	65.02 ± 0.15^{c}

Means with different superscript in a column indicate that values are significantly different.
MW1 = 100% maize; MW2 = 95% maize + 5% watermelon seed; MW3 = 90% maize + 10% watermelon seed; MW4 = 85% + 15% watermelon seed.

Mineral Element Composition of Maize-watermelon Seed Flour Blends

Table 3 shows that there were significant ($p<0.05$) increases in the mineral content of the flour blends which ranged from 114.00- 188.00 mg/100g for potassium, magnesium (412.00-672.00 mg/100g), copper (3.20- 4.90 mg/100g), and manganese (2.70- 6.60 mg/100g) However, calcium contents decreased from 748.00 mg/100g in the control sample to 588.00 mg/100g as watermelon seed flour increased in the blend. Previous studies have reported that masa by-products are potential source of both calcium and fibre for livestock diets (Rosentrater *et al*., 1999). Dehulled watermelon seed flour has been reported to contain magnesium (707.3 mg/100 g), potassium (918.3 mg/100 g), zinc (291.0 mg/100 g) and manganese (338.3 mg/ 100g) (Ifesan and Ebosele, 2017). Watermelon seeds are rich in mineral elements and could aid in digestion, formation of strong bones and teeth as well as hemoglobin synthesis and this will boost the mineral content of masa produced from the blends.

Table 3: Mineral Element Composition (mg/100g) of Maize–watermelon Seed Flour Blends

Samples	Calcium	Potassium	Magnesium	Copper	Manganese
MW1	748.00±2.83^{a}	114.00±2.83^{d}	412.00±2.83^{d}	3.20±0.42^{b}	2.70±0.28^{b}
MW2	674.00±2.83^{b}	156.00±4.24^{c}	444.00±2.83^{c}	3.90±0.42^{ab}	3.40±0.57^{b}
MW3	676.00±2.83^{b}	166.00±2.83^{b}	528.00±2.83^{b}	4.30±0.42^{ab}	3.50±0.42^{b}
MW4	588.00±2.83^{c}	188.00±2.83^{a}	672.00±2.83^{a}	4.90±0.28^{a}	6.60±0.57^{a}

Means with different superscript in a column indicate that values are significantly different.
Mw1 = 100% maize; MW2 = 95% maize + 5% watermelon seed; MW3 = 90% maize + 10% watermelon seed; MW4 = 85% + 15% watermelon seed.

Phytochemical and Antioxidant Properties of Maize-watermelon Seed Flour

Addition of watermelon seed flour to maize were found to have significantly ($p<0.05$) increase the antioxidant (DPPH), phytochemicals (phenols, flavonoids, tannins), and antinutrient (phytate with the exception of oxalate) (Table 4). The ability of maize-watermelon flour to scavenge free radicals (39.02%-60.74%) was observed to increase in the flour sample with the addition of watermelon seed. This may be attributed to the antioxidant activity reported for watermelon seed (Acar *et al.*, 2012; Tabiri *et al.*, 2016). Flavonoid content of raw maize was 621.02 mg/g and in the sample with 15% watermelon seed flour it has increased to 1526.24 mg/g, tannin (1.30 mg/g-3.00 mg/g) and phenol (604.76 mg/g-670.46 mg/g). Tannin and oxalate content of the blends were within the acceptable limit while the phytate content was higher than the recommended limit. It was however observed that maize flour has high amount of maize and addition of watermelon seed only contributed mild difference.

Table 4: Phytochemical and Antioxidant Property of Maize–watermelon Seed Flour Blends

Sample	Total Flavonoid Content (mgQE/g)	Tannin (mg/g)	Phenol (mgGAE/g)	Oxalate (mg/g)	Phytate (mg/g)	DPPH (%)
MW1	621.02±0.02^{d}	130.97±0.03^{d}	604.76±0.01^{d}	1.61±0.01^{a}	20.18±0.01^{d}	39.02 ± 0.02^{d}
MW2	942.75±0.01^{c}	169.03±0.03^{c}	637.64±0.02^{c}	1.54±0.01^{b}	20.37±0.01^{c}	39.90 ± 0.14^{c}
MW3	1149.72±0.02^{b}	258.09±0.00^{b}	652.37±0.02^{b}	1.42±0.03^{c}	20.49±0.02^{b}	55.98 ± 0.01^{b}
MW4	1526.24±0.02^{a}	314.3±0.01^{a}	670.46±0.02^{a}	1.36±0.01^{d}	20.61±0.02^{a}	60.74 ± 0.01^{a}

Means with different superscript in a column indicate that values are significantly different.

MW1 = 100% maize; MW2 = 95% maize + 5% watermelon seed; MW3 = 90% maize + 10% watermelon seed; MW4 = 85% + 15% watermelon seed.

Consumer Acceptability of Maize-watermelon Seed Masa

Table 5 shows the sensory attributes including taste, aroma, texture, appearance and overall acceptability of maize-watermelon seed masa samples as scored by the panelists. It was observed that for taste, texture and aroma, there were no significant differences ($p>0.05$) among the samples. Based on appearance, samples MW1, MW2 and MW3 were scored higher than masa sample with 15% watermelon seed. Masa samples supplemented with 5-10% watermelon seed were found to compare favourably with the control (100% maize). This result may mean that consumers of masa should be encouraged to supplement the maize with seeds rich in protein such as watermelon seed. About 10% addition may be considered.

Table 5: Sensory Attributes of Masa Produced from Maize-watermelon Seed Flour

Sample	Taste	Appearance	Aroma	Texture	Overall acceptability
MW1	5.93±1.49^{a}	6.46±2.00^{a}	6.07±1.03^{a}	6.13±1.92^{a}	6.87±1.51^{a}
MW2	6.53±1.25^{a}	7.00±1.07^{a}	5.93±1.10^{a}	6.13±1.12^{a}	6.73±0.88^{ab}
MW3	5.93±1.16^{a}	6.13±0.83^{a}	5.53±1.64^{a}	5.80±1.61^{a}	5.93±1.27^{ab}
MW4	5.76±1.53^{a}	4.93±2.15^{b}	5.67±1.91^{a}	5.00±1.80^{a}	5.67±1.80^{b}

Means with different superscript in a column indicate that values are significantly different.
Mw1 = 100% maize; MW2 = 95% maize + 5% watermelon seed; MW3 = 90% maize + 10% watermelon seed; MW4 = 85% + 15% watermelon seed.

Conclusion

Addition of watermelon seed to maize flour significantly increased the protein, fat, ash, mineral, some phytochemicals and DPPH free radical scavenging ability of the flour blends. Thus, masa from blends of maize-watermelon seed may offer a balanced diet to the consumer compared to masa from maize alone. There is need therefore to educate consumers on the health benefits of supplementing maize with protein sources that can also serve as functional food.

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